AACR-NCI-EORTC Virtual International Conference on
MOLECULAR TARGETS AND CANCER THERAPEUTICS
October 7-10, 2021

Treatment with the dual-mechanism ERK inhibitor, ASTX029, alters myeloid cell differentiation

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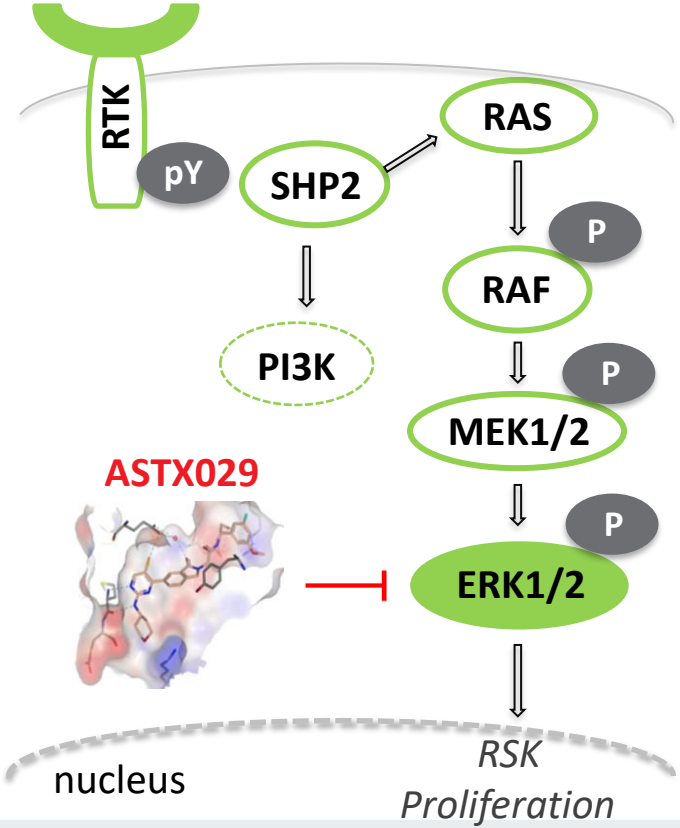
Disclosures

All authors are current employees of Astex Pharmaceuticals

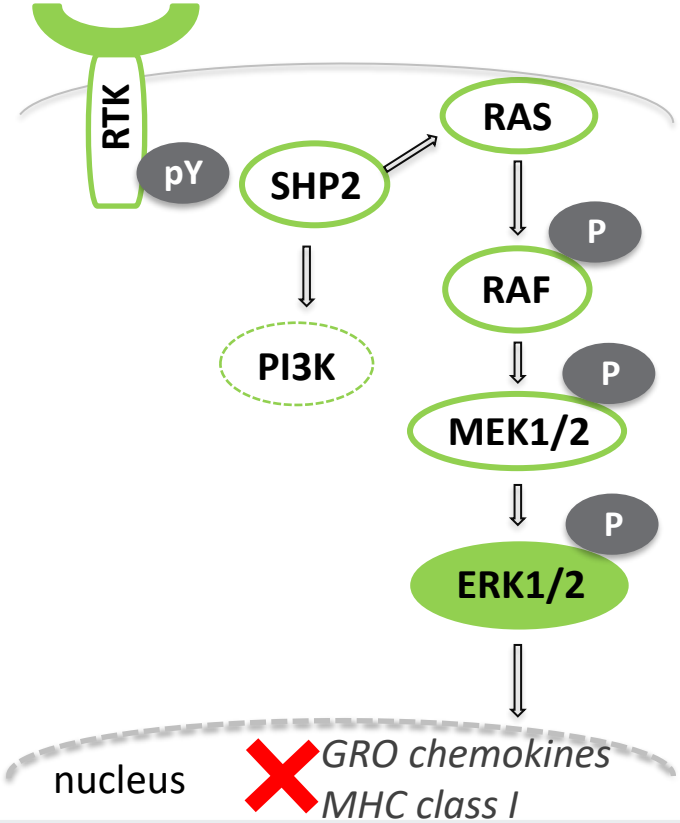
ASTX029: a dual-mechanism inhibitor of ERK1/2



- **We have recently reported* the discovery of ASTX029, a novel, potent and selective inhibitor of ERK1/2 with a dual-mechanism of action**
- **ASTX029 inhibits both the kinase activity of ERK and its phosphorylation by MEK1/2 and is a potent inhibitor of cell growth in MAPK-activated tumor models**

*Munck *et al.*, Mol Cancer Ther, 2021; Heightman *et al*., J Med Chem, 2021

ASTX029: a dual-mechanism inhibitor of ERK1/2



- **We have recently reported* the discovery of ASTX029, a novel, potent and selective inhibitor of ERK1/2 with a dual-mechanism of action**
- **ASTX029 inhibits both the kinase activity of ERK and its phosphorylation by MEK1/2 and is a potent inhibitor of cell growth in MAPK-activated tumor models**
- **There is growing evidence that activation of the MAPK pathway induces an immune-suppressive tumor microenvironment (TME) and inhibition of MAPK signaling results in a more pro-inflammatory TME**
- **Our previous data demonstrate that treatment with ASTX029 increases antigen presentation and cytokine signaling**

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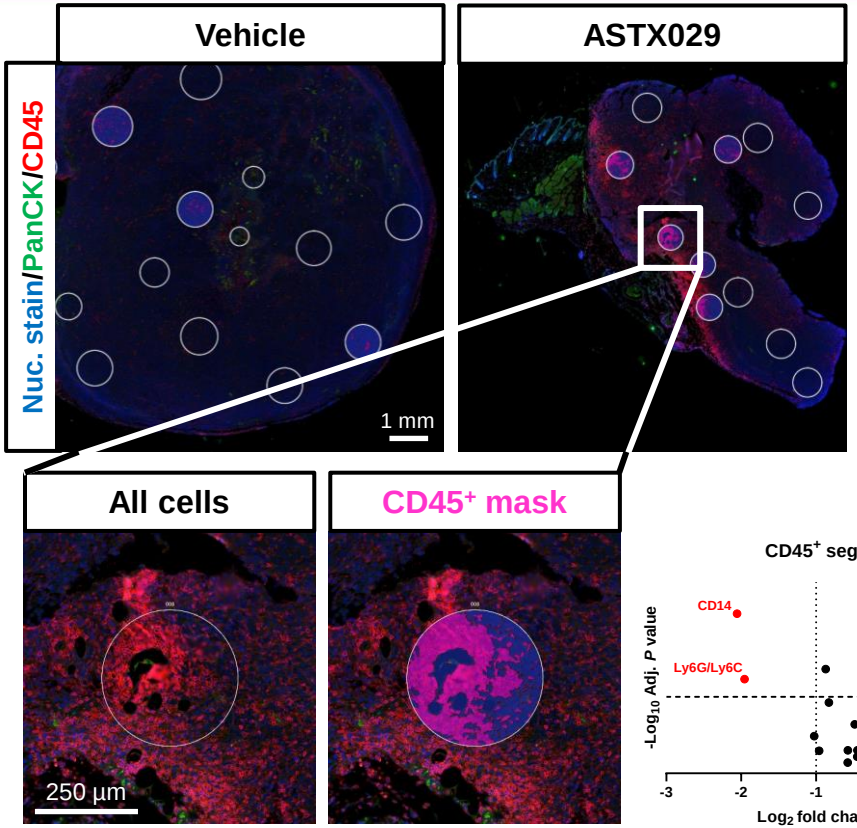
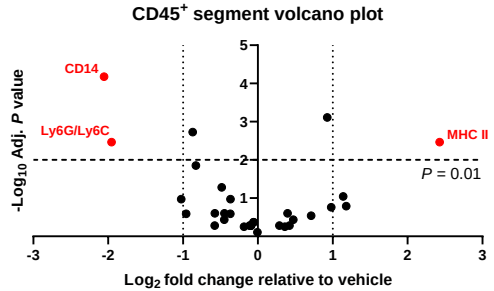
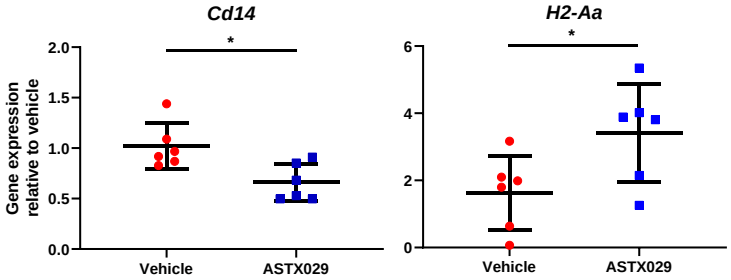
ASTX029 induces changes in immune cells in the TME in vivo

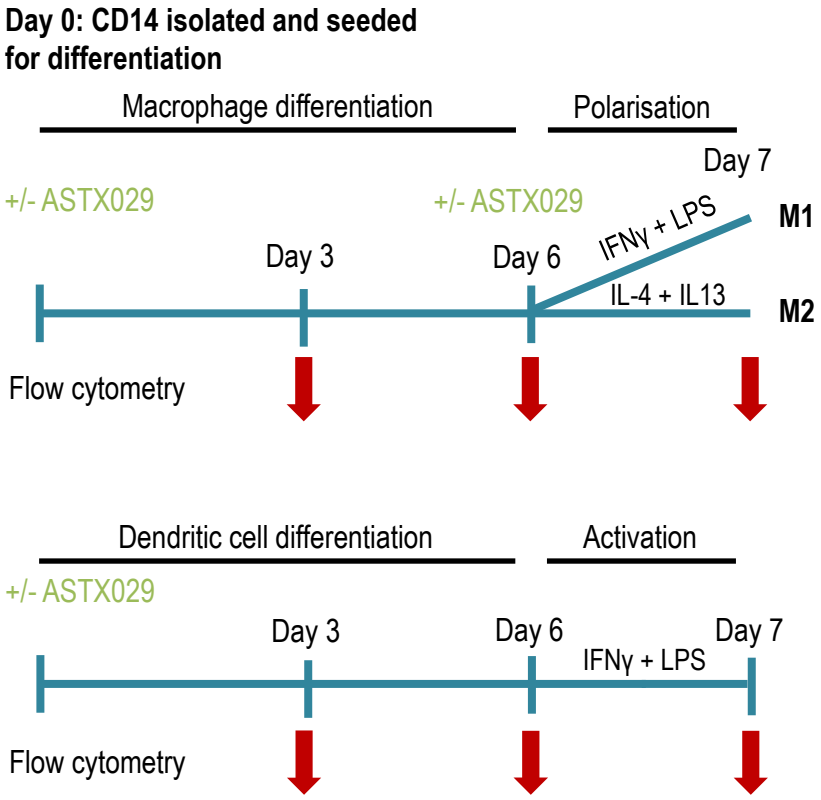


- **Profiling of the immune TME was performed in vivo in CT-26 syngeneic tumors treated for 6 days with vehicle or 75 mg/kg ASTX029**
- **Digital spatial profiling identified a significant decrease in CD14 and increase in MHC class II**
- **Changes confirmed by NanoString and qRT-PCR**
- **Is this due to the effect of ASTX029 on the TME directly or on the tumor?**



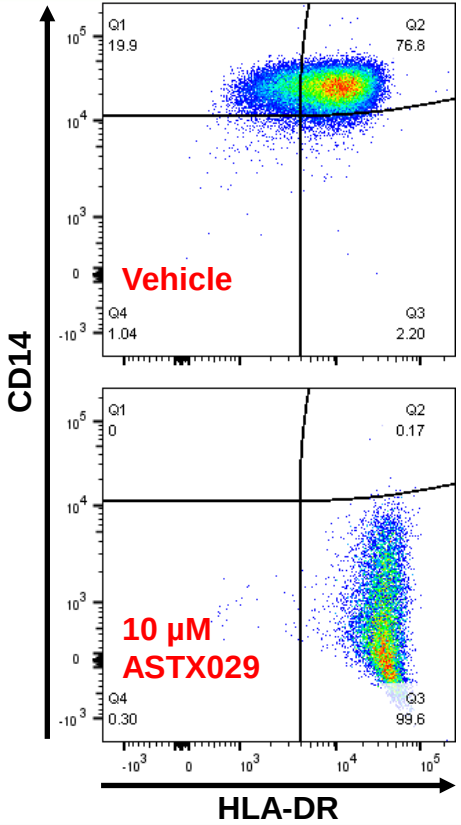


Experimental design for treatment of primary human myeloid cells



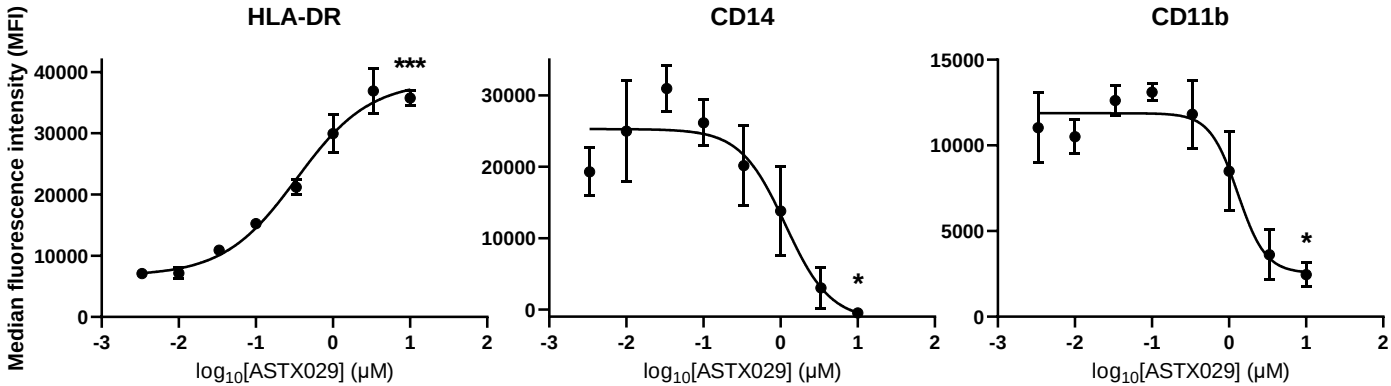
- CD14⁺ cells from 3 healthy donors were isolated from PBMCs and were differentiated into macrophages or dendritic cells
- Differentiated macrophages (Day 6) were polarized to M1 or M2 subtypes
- Immature dendritic cells (Day 6) were activated by addition of IFNγ + LPS
- ASTX029 was added either at the start of the differentiation process or immediately prior to macrophage polarization
- Samples were collected for flow cytometry on Days 3, 6 and 7

ASTX029 changes cell surface marker expression during macrophage differentiation from primary monocytes



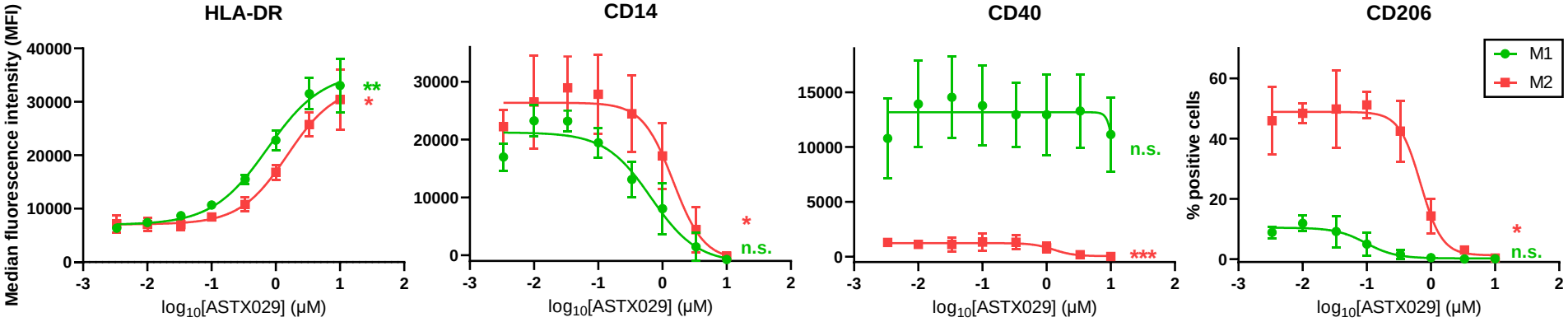
- **Changes in cell surface marker expression assessed on Day 6 of macrophage differentiation in presence or absence of ASTX029**
- **Significant increase in surface expression of HLA-DR (MHC class II) and decrease in CD14 and CD11b following treatment with 10 µM ASTX029**
  - Changes in cell surface markers are dose-dependent

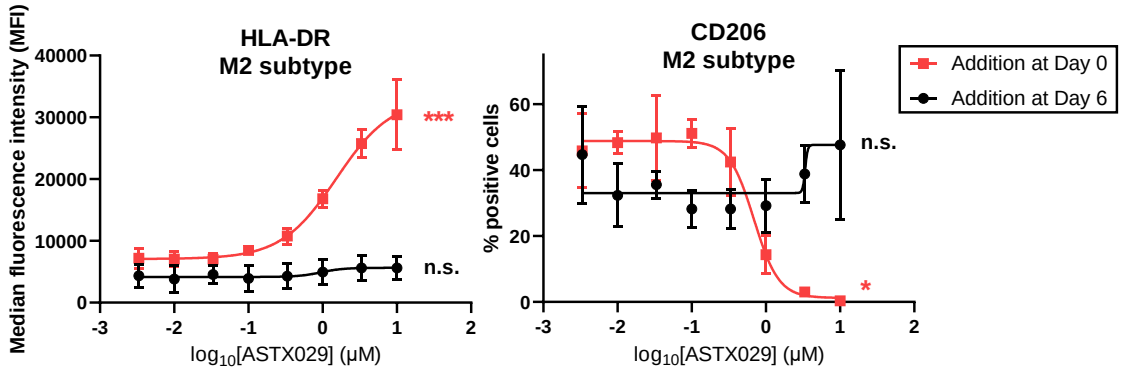
Dose-response of marker expression compared to vehicle controls on Day 6



All data points represent mean ± standard deviation, n=3 (*, p<0.05; ***, p<0.001; Welch's t-test)

ASTX029 alters macrophage marker expression in a subtype-specific manner



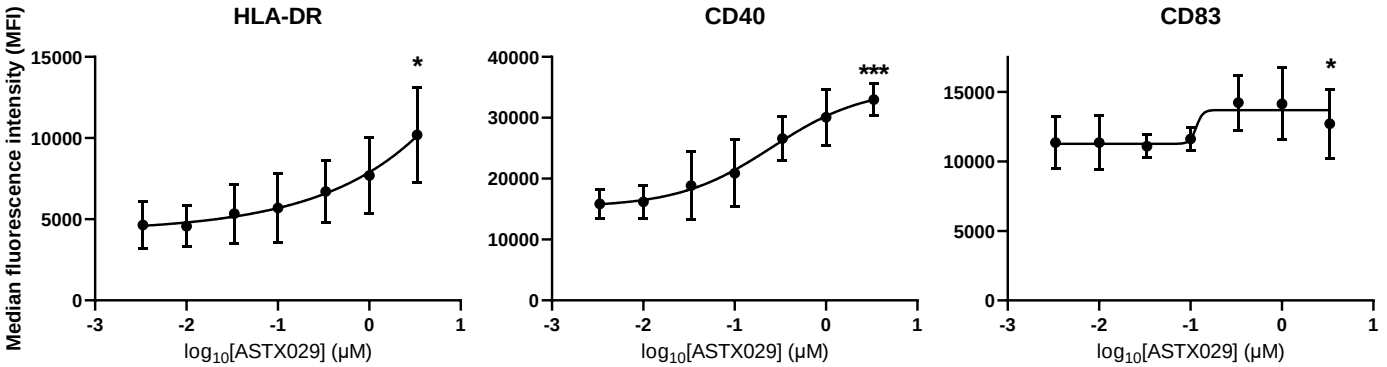


- **Subtype-specific changes in marker expression apparent after polarisation**
  - Significant reduction in CD14, CD40 and CD206 in M2 subtype only
- **Addition of ASTX029 just prior to polarisation does not alter markers**
  - Action on monocytes, not macrophages?

All data points represent mean ± standard deviation, n=3 (*, p<0.05; **, p<0.01; ***, p<0.001; Welch's t-test)

ASTX029 increases HLA-DR and dendritic cell markers of activation on primary cells

Change in cell surface marker expression on treatment with ASTX029 following activation at Day 7



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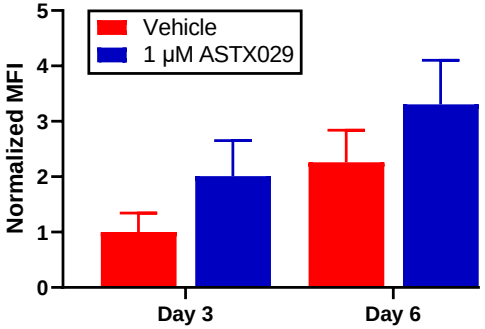
Comparison of CD80 expression at Day 3 and Day 6 of differentiation



- **Dose-dependent increase in markers of antigen presentation (HLA-DR) and T cell co-stimulatory molecules (CD40) on treatment with ASTX029**
  - Small but significant increase in CD83, a marker of dendritic cell maturation, on treatment with ASTX029
- **Trend towards increased speed of differentiation on treatment when comparing Day 3 and Day 6**

Summary

- **ASTX029 induces changes in the myeloid compartment in a syngeneic mouse model**
- **Profiling of ASTX029 using primary human in vitro-derived macrophages and dendritic cells demonstrates that ASTX029 has a direct effect on myeloid cell differentiation**
- **Treatment with ASTX029 upregulates HLA-DR expression on primary human differentiated macrophages and activated dendritic cells**
  - Future studies will investigate how treatment with ASTX029 changes T cell activation and tumor cell killing
- **ASTX029 is currently undergoing clinical development as part of a Phase 1/2 trial in advanced solid tumors (NCT03520075)**
- **Our data provide a rationale for the combination of ASTX029 with other agents, including those which modulate myeloid cell activation or signaling, to further enhance anti-tumor activity**